Running title: Age-specific reproductive investment

Age-specific reproductive investment in female burying beetles: independent effects of state and risk of death

Authors: S.C. Cotter[1,2], R. J. S. Ward[1] & R.M. Kilner[1, 3]

Address: [1] Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK

[2]School of Biological Sciences, Queen's University Belfast, MBC, 97 Lisburn Rd, Belfast

[3] Centre for Advanced Study, Drammensveien 78, NO-2071, Norway

Corresponding author: Dr. Sheena Cotter, School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Rd, Belfast, BT9 7BL. Tel: +44 (0)28 9097 2691, Fax: +44 (0)28 9097 5877. Email: s.cotter@qub.ac.uk

Summary

1. How much should an individual invest in reproduction as it grows older? Answering this question involves determining whether individuals measure their age as the time left for future reproduction or as the rate of deterioration in their state. Theory suggests that in the former case individuals should increase their allocation of resources to reproduction as opportunities for future breeding dwindle, and terminally invest when they breed for the last time. In the latter case they should reduce their investment in reproduction with age, either through adaptive reproductive restraint or as a passive by-product of senescence.

2. Here we present the results of experiments on female burying beetles, *Nicrophorus vespilloides*, in which we independently manipulated the perceived risk of death (by activating the immune system) and the extent of deterioration in state (by changing age of first reproduction and/or prior investment in reproduction).

3. We found that the risk of death and state each independently influenced the extent of reproductive investment. Specifically, we found a state-dependent decline in reproductive investment as females grew older that could be attributed to both adaptive reproductive restraint and senescence. A perceived increase in the risk of death, induced by activation of the immune system, caused females to switch from a strategy of reproductive restraint to terminal investment. Nevertheless, absolute reproductive investment was lower in older females, indicating constraints of senescence.

4. Our results show that a decline in reproductive investment with age does not necessarily constitute evidence of reproductive senescence but can also result from adaptive reproductive restraint.

5. Our results further suggest that the extent of reproductive investment is dependent on several different intrinsic cues and that the particular blend of cues available at any given age can yield very different patterns of investment. Perhaps this explains why age-related reproductive investment patterns seen in nature are so diverse.

Keywords: Ageing, immunity, life history strategy, parental care, reproductive restraint, senescence, state-dependent, terminal investment

Introduction

How much should an individual invest in reproduction as it grows older? Explaining age-specific patterns of fecundity is a fundamental goal of life history theory, yet observations of natural populations suggest that there is no simple answer to this question. In some species, such as California gulls *Larus californicus* (Pugesek, 1981) and collared flycatchers *Ficedula collaris* (Pärt *et al.*, 1992), reproductive investment increases with age, whereas in others, such as red-billed choughs *Pyrrhocorax pyrrhocorax* (Reid *et al.*, 2003) and red deer *Cervus elaphus* (Nussey *et al.*, 2008), it declines. Within these broad-scale population-level trends, there is additionally considerable variation among individuals in their age-specific schedules of fecundity (e.g. McElligott *et al.*, 2002; Nussey *et al.*, 2008; Tavecchia *et al.*, 2005).

Two sorts of theoretical analysis have been advanced to explain the way in which organisms schedule their reproduction with age (see also Broussard *et al.*, 2005). Classical life history models (Gadgil *et al.*, 1970) predict that reproductive investment should increase with age, peaking in an act of terminal investment when there are no further opportunities for reproduction (Clutton-Brock, 1984; Hirschfield *et al.*, 1975; Williams, 1966). A key assumption of this theoretical approach is that individuals can predict when the end is nigh and can base the extent of their reproductive investment on information about the time they have left for further breeding (McNamara *et al.*, 1996). A major shortcoming, however, is that it fails to capture the age-related variation in numerous dimensions of quality shown by real animals that is likely to influence the extent of investment in reproduction. This is better described by state-dependent life history theory, which assumes that reproductive investment decisions depend on one or more measures of condition, each of which may change at a

different rate as an individual grows older (McNamara *et al.*, 1996). Rather than seeking the best level of reproduction given the future likelihood of breeding before death, as in the classical life history models, state-dependent models instead find the best level of reproductive investment for an individual's state. In general, their predictions differ from classical life history theory. The reproductive restraint model (McNamara *et al.*, 2009), for example, assumes that reproduction contributes nonlinearly to the rate at which the body deteriorates. Under these conditions, selection favours individuals that practise reproductive restraint in later life because this slows the rate of their decline, effectively buying them more time with which to increase their lifetime reproductive success (McNamara *et al.*, 2009). More general hypotheses for senescence (Rose, 1991) make the same prediction as the reproductive restraint hypothesis but for a different reason. Here the suggestion is that older individuals are so worn out or damaged or lacking in resources (e.g. Cichon, 2001; Kirkwood *et al.*, 1991; Monaghan *et al.*, 2009; Partridge, 2010; Partridge *et al.*, 2006) that they are simply incapable of investing very much in reproduction in late life.

Understanding whether reproductive investment should increase or decrease with age therefore first involves determining whether individuals are using cues about the risk of their own death or the rate of deterioration in their state to inform their breeding (McNamara *et al.*, 1996; McNamara *et al.*, 2009). In the former case, they should increase their reproductive investment as they get older whereas in the latter case, investment levels should fall. It is possible to distinguish these two alternatives empirically because the risk of death can be manipulated independently of state by varying extrinsic cues about the likelihood of predator- or pathogen- induced mortality. Nevertheless, to our knowledge, no empirical work has yet done this, nor has the recently proposed reproductive restraint hypothesis (McNamara *et al.*, 2009) yet been subjected to empirical testing.

Here we use experiments on the burying beetle *Nicrophorus vespilloides* (Figure 1) to determine whether the body's state or the risk of death best explains the extent of reproductive investment with age. To breed, burying beetles must first locate the carcass of a small vertebrate, and these become available at unpredictable intervals (Eggert *et al.*, 1997; Scott, 1998). The burying beetle is therefore an opportunistic breeder and hence potentially experiences highly variable levels of damage accumulation in nature before it breeds for the first time. This means that reproductive decisions are likely to be sensitive to state, making it ideal for this sort of investigation (see also Lock *et al.*, 2007, and Creighton *et al.*, 2009; Trumbo, 2009 for work on *N. orbicollis*). Consistent with this idea, when burying beetles are allowed to breed with a super-abundance of carcasses under relatively benign laboratory conditions, their fecundity declines as they grow older (Creighton *et al.*, 2009; Ward *et al.*, 2009).

The unpredictability of carcass arrival in nature also means that burying beetles cannot infer from the passage of time alone whether or not their first breeding attempt will also be their last, and so must rely on other cues to infer the risk of death. The scarcity of carcass availability triggers intense competition among rival beetles, who then fight viciously to secure this key breeding resource (Trumbo, 1991). Burying beetles are thus vulnerable to opportunistic infections arising from fight wounds, because they live in a microbe-rich soil environment (Plaistow *et al.*, 2003). Consequently, they might rely on the state of their immune system to infer the risk of death, just as other species have been shown to do. Species as diverse as house sparrows *Passer domesticus* (Bonneaud *et al.*, 2004), Siberian hamsters *Phodopus sungorus* (Weil *et al.*, 2006), mealworm beetles *Tenebrio molitor* (Sadd *et al.*, 2006) and crickets *Acheta domesticus* (Adamo, 1999) all increase their investment in reproduction when their immune system is activated, and this is typically interpreted as

evidence of terminal investment in response to a cue that the risk of death is very high (e.g. Velando *et al.*, 2006). Importantly, previous work shows that activation of the immune system alone is sufficient to induce terminal investment: the trigger for immune system activation itself can be benign and have no measurable influence on the subject's lifespan (Bonneaud *et al.*, 2004; Sadd *et al.*, 2006; Velando *et al.*, 2006; Weil *et al.*, 2006). Burying beetles might similarly use their infection status to gauge their risk of death, and therefore how much they should invest in reproduction.

The experiments we describe here manipulate both the rate of deterioration in state (by varying the extent of prior investment in survival and reproduction) and the perceived risk of death (by activating the immune system). In our first experiment, we experimentally decouple age and state to determine their independent effects on current reproductive investment. Classical life history theory predicts that reproductive investment should increase with age whilst state-based hypotheses predict that investment should fall (McNamara *et al.*, 2009). Experiment 1 thus allows us to determine whether classical life history theory or state-based life history better explains age-related reproductive investment in the burying beetle, but it cannot distinguish senescence from reproductive restraint. This is the aim of our second experiment. Although the predictions of the reproductive restraint and senescence hypotheses seem alike, in the former case, the extent of investment in reproduction is the consequence of strategic choice while in the latter, it is determined by constraint. In experiment 2 we attempt to distinguish the two hypotheses by testing whether we can enforce an alternative strategic choice or whether the extent of reproductive investment is simply constrained by senescence. Specifically, we create the illusion of an increased risk of death by giving young and old females a non-pathogenic immune challenge and we measure their consequent reproductive investment. Classical life history theory predicts that reproductive

147 investment should increase if the risk of death is suddenly increased. The magnitude of
148 change, and the absolute level of terminal investment thus induced, are key to distinguishing
149 reproductive restraint from senescence. If older females increase the extent of their
150 investment to a similar or greater degree than younger females, then they must previously
151 have been exhibiting some form of reproductive restraint. If the increase in their investment
152 is lower than seen in younger females, or remains unchanged, then their patterns of
153 investment are constrained by senescence.

154

155 ## Materials and Methods

156 ***Study species***

157 *Nicrophorus vespilloides* uses resources on a small vertebrate carcass to nourish
158 young. Having secured a carcass, the pair typically prepare it for breeding by stripping the
159 body of fur or feathers and interring it in a shallow grave. During this time, the female also
160 lays her eggs in the soil near the carcass. Two to three days later, the larvae hatch and crawl
161 to the carcass, where they take up residence. There the offspring may be fed and defended by
162 both parents (Eggert *et al.*, 1997; Scott, 1998), although female-only care is also seen in
163 nature (Muller *et al.*, 2007). In the laboratory, widowed females fully compensate for the
164 absence of males when caring for larvae (Smiseth *et al.*, 2005). We know from our previous
165 work that by the time the larvae come to disperse, roughly eight days after hatching, their
166 weight is an accurate indication of their parents' reproductive investment (Ward *et al.*, 2009).
167 Beetles become reproductively active approximately 10 days after emerging as adults and
168 their fecundity declines dramatically after approximately 60 days (SCC pers obs).

169

N. vespilloides colony

The *Nicrophorus vespilloides* colony was established in May 2005 from wild-caught beetles which had been trapped in Madingley Woods, Cambridge, UK. Wild beetles were collected from Byron's Pool local nature reserve, Cambridge, UK each subsequent year during August and added to the colony to maintain genetic diversity. Beetles were reared in a temperature controlled room at 21°C with a 16:8 light:dark cycle. Unrelated pairs were placed in a plastic container (17 x 12 x 6 cm), one-third filled with moist, non-sterile soil, and provided with a newly defrosted mouse carcass (10.82 ± 0.2 g). After carcass preparation, we removed males from the breeding box, so that the subjects of our experiments were widowed females. The breeding box was kept in the dark to simulate underground conditions. At dispersal, larvae were removed from the soil and placed individually in plastic boxes (12 x 8 x 2 cm) filled with moist soil. Upon reaching adulthood, beetles were maintained in their individual containers and fed twice a week on small pieces of minced beef until required for experiments or breeding. Between 50 and 150 pairs successfully produced offspring each generation. Animals had been reared under standard laboratory conditions for 18 generations at the start of the experiment.

Experiment 1: Manipulating deterioration in state by varying age at first reproduction and the extent of prior investment in reproduction

We know from our previous experiments that investment in survival and investment in reproduction each cause female state to deteriorate (Ward, 2007; Ward *et al.*, 2009). However these two traits are usually strongly correlated; in unmanipulated beetles, where females are allowed to breed from the onset of maturity until death, the correlation between

age and prior reproductive investment is extremely high ($R = 0.80$, $t_{131} = 15.06$, $P < 0.001$; unpublished data), meaning that it would be difficult to assess their effects on current reproduction independently. The aim of this experiment was to create females for whom investment in survival and investment in reproduction were not correlated. This we achieved through the independent manipulation of age at first reproduction and the extent of prior investment in reproduction (see Table 1 for details of experimental design). It also allowed us to test whether reproduction accelerates deterioration in state caused by investment in survival alone, which is a key assumption of the reproductive restraint hypothesis (McNamara *et al.*, 2009).

Virgin females were mated and allowed to raise young for the first time at 12, 24, 36, 48 or 60 days post-eclosion. Following this first bout of reproduction females were remated and allowed to raise young every 12 days until death, therefor each female was mated between 1 and 5 times. Females were given the opportunity to breed irrespective of whether their previous bout(s) had been successful or not. Between breeding bouts females were returned to their individual containers. The experiment was carried out over 6 blocks with 75, 59, 40, 40, 40 and 144 females in each block, giving 398 females in total. Only 4 females produced offspring at 72 or 84 days of age therefore we did not analyse reproductive investment after 60 days of age due to inadequate sample sizes at these later time points.

a) Effect of age at first reproduction

To investigate the effect of age at first reproduction on reproductive investment, we analysed data collected from first broods only (Table 1). For each age class, we recorded the

percentage of females that bred successfully. The weight of the brood produced by successful breeders was also measured.

b) Effect of age at first reproduction and the extent of prior investment in reproduction

This analysis used data collected from all broods produced by females mated for the first time at 24, 36, 48 or 60 days post-eclosion (Table 1). We considered how current reproductive investment in successful broods was affected by age and prior reproductive investment, the latter measured as the combined weight of larvae produced in previous reproductive bouts. Since we manipulated age of reproduction independently of prior investment in reproduction, the two variables were only weakly correlated ($R = 0.32$, $t_{578} = 8.05$, $P < 0.001$) and we were able tease apart their effects statistically. This allowed us to test whether prior reproductive investment accelerated survival-related deterioration in state. Females breeding at age 12 were not included in this analysis as all females of this age were virgins, meaning that age and prior reproductive investment were collinear for this age group.

Experiment 2: Joint manipulation of deterioration in state and perceived risk of death

a) Developing an appropriate protocol

Non-pathogenic immune challenges have routinely been used in both vertebrate and invertebrate studies to create the illusion of an increased risk of death (see Introduction), although never before with burying beetles. To mimic previous work on other species, our first goal was to develop a way to activate an immune response that did not reduce beetle survival directly. Seventy adult beetles were cleaned with ethanol and then pierced behind the

pronotum with a needle that was either sterile, or that had been dipped in dead bacterial cells (*Micrococcus lysodeikticus*). Beetles were then placed back into their containers with fresh food for 24 h. Haemolymph samples were collected from half of the beetles using a drawn capillary tube and blown into an Eppendorf tube. Haemolymph was successfully collected from 30 of the 35 beetles sampled, 15 control and 15 challenged, the remaining 5 beetles were removed from the analysis. This allowed us to test the independent effects of immune challenge and haemolymph sampling on the survival of the beetles. The antibacterial activity of the beetles' haemolymph in response to immune challenge was assessed by quantifying its ability to inhibit the growth of live bacteria. Bacterial test plates were made up the day before haemolymph collection using an agar overlay technique with *M. lysodeikticus* as described in (Povey *et al.*, 2009). In each plate 20 holes were punched in the agar using a capillary tube. Two 1 µl replicates of each haemolymph sample were pipetted into the labelled holes on the agar plates. The plates were incubated for 24 h at 37°C and antibacterial activity was measured as the diameter of the clear zone of bacterial inhibition around the holes in the plate. The diameter of the clear zones was calculated using Image J software (http://rsweb.nih.gov/ij/index.html). All beetles, those that had their haemolymph sampled and those that had not, were then returned to their containers and fed twice weekly until death and the date of death recorded.

b) Experimental activation of the immune system

For the main experiment we activated the immune system of both young (average age 23.1 days ± 0.37) and old (average age 43.5 days ± 0.38) virgins, reasoning from the results of Experiment 1 that older beetles would have experienced a greater deterioration in state

than younger individuals. Note that in this experiment, all of the beetles were virgins so there was no additional decrease in state attributable to prior reproductive investment. Beetles in each age class were randomly assigned to one of two immune challenge treatments: control beetles were pierced with a sterile needle (control) whilst challenged beetles were pierced with a needle dipped in dead *M. lysodeikticus* cells (challenged).

Each female was weighed, pierced with a sterile or bacteria-dipped needle, then placed in a breeding box with an unrelated virgin male and a mouse carcass (mean weight (SE) = 13.89g (0.51)) for breeding. Males were removed after 2 days, before larvae hatched, so that focal females reared the brood alone. After 7 days each box was checked for dispersing larvae, females were weighed and larvae counted and weighed. The experiment was carried out in 4 blocks with 65, 32, 48 and 61 pairs in each block, giving 206 pairs in total, 104 in the control treatment group and 102 in the immune challenged treatment group.

Statistical analyses

In both experiments the likelihood of breeding data were analysed using the logistic regression function with the logit link in SPlus 7 (Tibco software, Palo Alto, California). Where the residual variance and residual df were approximately equal chi squared tests were used to test the significance of the terms in the models (Wilson *et al.*, 2002). The age at first breeding data in experiment 1a and the data in experiment 2 were analysed using GLM. The data from experiment 1b was analysed using linear mixed effects REML models in Genstat 10 (VSN International, Hemel Hempstead, UK) with female ID included as a random effect. In experiment 1, age, block and prior reproductive investment were included as fixed effects in the models. In experiment 2, carcass weight, female weight, block, age and immune-challenge treatment were included as fixed effects. Block was included as a fixed effect

rather than a random effect as the number of blocks used (6) was too small to accurately estimate the mean and variance of the population from which they were randomly drawn (R. Knell pers. com.). In each case the total weight of the current brood was used as the measure of reproductive investment. However, using the number of larvae as the measure of reproductive investment gave very similar results (data not shown). Means ± standard errors are reported throughout.

Results

Experiment 1: Manipulating deterioration in state by varying age at first reproduction and the extent of prior investment in reproduction

a) Effect of age at first reproduction

The total weight of offspring that females produced at first reproduction declined with age (GLM: $F_{4,189} = 6.37$, $P < 0.001$; Fig. 2). An examination of the means and standard errors suggested that neither ages 24 and 36, or 48 and 60 appeared to be significantly different from each other. The analysis was rerun with three age groupings, young (12 days), medium (24 and 36) and old (48 and 60) this model did not differ significantly from the model with 5 age groups (likelihood ratio = -3.83, df = 1, $P = 0.05$; Fig. 2). However, grouping ages 12, 24 and 36 as young beetles and 48 and 60 as old beetles gave a significantly worse fit than the 3 age groups (likelihood ratio = -1.36, df = 2, $P = 0.51$; Fig. 2), therefore 3 age groupings represented the best fit to the data.

Age at first reproduction did not affect the likelihood that females would successfully rear a brood (logistic regression: $\chi^2_4 = 5.63$, $P = 0.23$), but there was a trend for young

306 females to be more likely to breed than old females (logistic regression grouping ages 12-36
307 vs 48-60: $\chi^2_1 = 2.98$, $P = 0.08$).

308 **b) Effect of age at reproduction and the extent of prior investment in reproduction**

309 The age-related decline in reproductive investment occurred only in older females that
310 had previously invested in reproduction: we found a significant interaction between age at
311 reproduction and the extent of prior reproductive investment (age*prior investment: $F_{3,245} =$
312 6.67, $P < 0.001$, Fig. 3). It can be seen from the figures that the negative effects of prior
313 reproductive investment on current reproduction were felt in older females only (Fig. 3b-d).

314

315 **Experiment 2: Joint manipulation of deterioration in state and perceived risk of death**

316 **a) Developing an appropriate protocol**

317 Antibacterial activity was upregulated following immune challenge with dead
318 bacterial cells (Mean diameter of clear zone in mm: Control = 6.84 $\pm$ 0.86, Challenged =
319 10.00 $\pm$ 0.68; $F_{1,28} = 8.15$, $P = 0.008$). There were no effects of the immune challenge
320 treatment ($F_{1,63} = 0.01$, $P = 0.92$) or haemolymph sampling on survival ($F_{1,62} = 1.22$, $P =$
321 0.27).

322

323 **b) Experimental activation of the immune system**

324 *Effect of the immune challenge (perceived risk of death)*

325 After accounting for block effects age significantly reduced the likelihood that
326 females would breed successfully irrespective of the treatment the females received (logistic

regression; age*treatment: $\chi^2_1 = 3.54$, $P = 0.06$; age: $\chi^2_1 = 19.75$, $P < 0.001$; treatment: $\chi^2_1 = 0.15$, $P = 0.70$). However, irrespective of the age of the female, the immune challenge treatment caused breeding females to increase the weight of their current brood ($F_{1,113} = 4.93$, $P = 0.028$; Fig. 4a). Block was not significant and so was removed from the model ($F_{1,110} = 1.32$, $P = 0.271$). Neither female weight ($F_{1,93} = 0.86$, $P = 0.35$) or carcass weight influenced the weight of the brood ($F_{1,92} = 0.01$, $P = 0.91$).

Reproduction was costly for females in terms of their change in body weight ($F_{1,140} = 18.31$, $P < 0.001$). Females that chose not to breed gained on average 16 ± 5 mg, whilst females that produced a brood lost on average, 1 ± 5 mg. Of the females that did breed, their weight change was not affected by their age ($F_{1,86} = 0.01$, $P = 0.99$), but was negatively affected by both the weight of the brood they had produced ($F_{1,86} = 5.62$, $P = 0.02$; effect = 45 ± 18) and the immune challenge treatment ($F_{1,86} = 6.67$, $P = 0.01$; Fig. 4b).

Effect of age (state)

Irrespective of immune treatment (treatment*age: $F_{1,110} = 0.53$, $P = 0.47$), older females produced lighter broods (age: $F_{1,113} = 9.71$, $P = 0.002$; Fig. 4a), just as in Experiment 1. Age also influenced the likelihood that females would reproduce successfully, with older females being significantly less likely to produce a brood after accounting for block effects (binomial model; age: $\chi^2_1 = 18.45$, $P < 0.0001$, treatment: $\chi^2_1 = 0.15$, $P = 0.70$; treatment*age interaction: $\chi^2_1 = 3.40$, $P = 0.067$).

Discussion

Theoretical work suggests that the key to understanding intra- and inter- specific variation in age-specific fecundity lies in establishing whether investment in current reproduction is determined by the time left for future reproduction or by the body's deterioration in state (McNamara *et al.*, 2009). We have shown that each independently affects the extent of reproductive investment in female burying beetles. By varying prior investment in survival and/ or reproduction, we experimentally decreased female state to differing degrees and found a corresponding decline in their reproductive investment with age (Fig. 2; see also Ward *et al.*, 2009). Furthermore, we also found that extensive prior investment in reproduction accelerated this decline in older beetles (Figs. 3b-d), just as is assumed by the reproductive restraint hypothesis (McNamara *et al.*, 2009).

When we challenged the burying beetle's immune system, and created the illusion that the likelihood of breeding again was very low for some individuals (e.g. Bonneaud *et al.*, 2004; Velando *et al.*, 2006), all breeding females, whether young or old, produced heavier broods, just as predicted by classic life history theory (Fig. 4a). Females are therefore clearly capable of investing far more than we otherwise see, which indicates that they are typically strategically refraining from investing in reproduction, again just as described by the reproductive restraint hypothesis. We cannot identify the nature of this reproductive restraint from our data: they may be laying fewer eggs, or cannibalizing larvae to keep investment levels down. We are confident that by activating the immune system we created the illusion of an increased risk of death, and that there is no ready alternative explanation for our results. We are not the first to find that a non-pathogenic immune challenge increases the extent of reproductive investment (Bonneaud *et al.*, 2004; Sadd *et al.*, 2006; Velando *et al.*, 2006; Weil *et al.*, 2006; but see Reaney *et al.*, 2010), and we are following a well established precedent

372 in the literature in concluding that activation of the immune system in this way induces
373 individuals to believe they have reduced survival prospects (Bonneaud *et al.*, 2004; Sadd *et*
374 *al.*, 2006; Velando *et al.*, 2006; Weil *et al.*, 2006).

375 Although older and younger females increased their investment to a similar degree
376 when immune challenged, absolute levels of investment were consistently lower in older
377 females (Fig. 4a), suggesting that they were additionally constrained in the extent of their
378 reproductive investment because senescence limits fecundity as females age. In addition,
379 older females were less likely to attempt to breed than younger females, which might also be
380 explained by the effects of senescence. Intriguingly, the latter results are the exact opposite of
381 those obtained on the related burying beetle *N. orbicollis* (Trumbo, 2009). The reason for this
382 discrepancy in findings is unclear but it may be driven by differences in population density
383 experienced by females prior to breeding. In our study, females were reared in isolation,
384 whereas in Trumbo's (2009) study, females were reared in groups. *N. orbicollis* has been
385 shown to tailor its levels of reproductive investment to both population density and seasonal
386 cues (Scott 1998).

387 In short, our experimental work therefore provides support for classical life history
388 models (Clutton-Brock, 1984; Gadgil *et al.*, 1970; Hirschfield *et al.*, 1975; Williams, 1966),
389 state-dependent life history models (McNamara *et al.*, 1996; McNamara *et al.*, 2009) and
390 hypotheses for senescence (e.g. Cichon, 2001; Kirkwood *et al.*, 1991; Monaghan *et al.*, 2009;
391 Partridge, 2010; Partridge *et al.*, 2006). It also indicates that evidence of a decline in
392 reproductive investment with age may be the consequence of reproductive restraint rather
393 than senescence, though the latter is the typical interpretation in the literature (e.g. Broussard
394 *et al.*, 2005; Creighton *et al.*, 2009; Nussey *et al.*, 2008; Reid *et al.*, 2003; Velando *et al.*,
395 2006).

The most parsimonious interpretation of our results is that throughout their lives individuals balance investment in reproduction against investment in survival, holding back resources from each reproductive attempt to prolong survival and therefore increase the number of lifetime breeding opportunities (McNamara *et al.*, 2009). Our experiments show that the primary intrinsic cue used by burying beetles for assessing their survival prospects is some measure of state. When an individual has a high state then investment in survival is always prioritized over investment in reproduction, but when survival prospects are compromised, as indicated by a challenged immune system for example, individuals then allocate resources away from survival and dramatically increase their reproductive investment.

By using a combination of state-based and mortality risk-related cues to determine the extent of investment in reproduction, female burying beetles may optimize their age-specific fecundity even though their opportunities for reproduction are based on an uncertain availability of breeding resources. We suggest that a female's default strategy is to assume that times will be as good as they possibly can be (*cf* (Lack, 1947), with a regular supply of carcasses for which there is little competition. We know from our previous work that female burying beetles can produce more than five broods during their life under these conditions, but that the number and quality of offspring that they rear gradually declines as females get older (Ward *et al.*, 2009); see also (Creighton *et al.*, 2009). The results we present here suggest that this decrease in fecundity is in part a consequence of reproductive restraint (McNamara *et al.*, 2009), because females are capable of producing larger broods than they actually do (Fig. 4a). By withholding resources from breeding, females can limit the deterioration in state caused by reproduction (Fig. 3), and buy the time to wait for a new carcass and a further breeding attempt.

If conditions rapidly take a turn for the worse, and the supply of corpses for breeding becomes more limited, then competition for carcasses intensifies, and the likelihood of sustaining an infection from a fight injury rises accordingly. A challenged immune system tells females that the risk of death is high (e.g. Bonneaud *et al.*, 2004; Velando *et al.*, 2006) and, with no further opportunities for reproduction, they facultatively switch from reproductive restraint to terminal investment. Nevertheless, even under these circumstances, older females produce smaller broods than younger ones (*cf* Creighton *et al.*, 2009), perhaps because senescence prevents them from investing more (e.g. Cichon, 2001; Kirkwood *et al.*, 1991; Monaghan *et al.*, 2009; Partridge, 2010; Partridge *et al.*, 2006). Whether males practice a similarly flexible age-related reproductive strategy remains to be determined, and it would be interesting to investigate whether it differs at all from that shown by females (Bonduriansky *et al.*, 2008; Nussey *et al.*, 2008).

It is likely that other species also use a diversity of cues to adjust their investment in reproduction as they grow older. For example, the results of an experimental study of male blue-footed boobies *Sula nebouxii* are remarkably similar to those we report here. Just as with female burying beetles, male blue-footed boobies typically show reproductive restraint in later life but can be induced to exhibit terminal investment when immune-challenged (Velando *et al.*, 2006). Although taxonomically distant, blue-footed boobies and burying beetles have broadly similar reproductive ecologies in that they each breed iteroparously and opportunistically (Nelson, 2005). Perhaps these similarities explain why they each respond to state- and time-based cues when adjusting their reproductive investment with age. Opportunistic breeders differ from seasonal breeders because the extent of their prior reproductive investment, and the consequent deterioration in state, is unpredictably correlated with the passage of time: over two years a seasonal breeder will certainly reproduce twice, for

example, but an opportunistic breeder may breed more than twice, or not at all, or some number in between. In opportunistic breeders, age and state will therefore be more weakly correlated than in seasonal breeders (McNamara *et al.*, 1996), which may explain why they employ additional cues, besides their state, when adjusting the extent of their reproductive investment.

Alternatively, perhaps state-based cues and cues relating to the time left for future reproduction, in conjunction with extrinsic ecological factors such as climate (e.g. Tavecchia *et al.*, 2005), density dependence (e.g. Kruuk *et al.*, 1999) or resource availability (e.g. McElligott *et al.*, 2002), are used routinely to determine the extent of reproductive investment with age in a diversity of species. The particular blend of cues used may vary among individuals, among populations and among species, perhaps because cue reliability itself is highly variable. For example, the status of the immune system may provide better information about the time left for future reproduction when the prevalent pathogens are more virulent than benign. Alternatively, or in addition, some individuals may be entirely insensitive to ecological information concerning the proximity of death (e.g. Marrow *et al.*, 1996). In general, the diversity of cues that individuals use to adjust their schedule of reproduction could account for the diversity of age-specific reproductive strategies seen in nature (Reaney *et al.*, 2010). The main message from our experimental work is that to understand how individuals invest in reproduction as they grow older, the challenge is to predict which cues are most likely influence strategic investment in fecundity and at what age their effect will be greatest.

Acknowledgments

This work was supported by the Natural Environment Research Council (NER/S/A/2004/12242 to RJSW); The Leverhulme Trust (PLP/2006/0033 to RMK); The Royal Society; The Isaac Newton Trust; and the Department of Zoology, University of Cambridge, UK. We are very grateful to Per Smiseth and Allen Moore for their logistical help and support in getting this project started and to Steve Trumbo, Rob Knell and an anonymous referee for their constructive and thought-provoking comments on an earlier draft of this paper. Author contributions: SCC and RMK designed the experiments, SCC and RJSW collected the data, SCC analysed the data, RMK and SCC co-wrote the paper.

References

Adamo, S.A. (1999) Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. *Animal Behaviour*, **57**, 117-124.

Bonduriansky, R., Maklakov, A., Zajitschek, F., & Brooks, R. (2008) Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional Ecology*, **22**, 443-453.

Bonneaud, C., Mazuc, J., Chastel, O., Westerdahl, H., & Sorci, G. (2004) Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the house sparrow. *Evolution*, **58**, 2823-2830.

Broussard, D.R., Michener, G.R., Risch, T.S., & Dobson, F.S. (2005) Somatic senescence: evidence from female Richardson's ground squirrels. *Oikos*, **108**, 591-601.

Cichon, M. (2001) Diversity of age-specific reproductive rates may result from ageing and optimal resource allocation. *Journal of Evolutionary Biology*, **14**, 180-185.

Clutton-Brock, T.H. (1984) Reproductive effort and terminal investment in iteroparous animals. *American Naturalist*, **123**, 212-229.

Creighton, J.C., Heflin, N.D., & Belk, M.C. (2009) Cost of reproduction, resource quality, and terminal investment in a burying beetle. *American Naturalist*, **174**, 673-684.

Eggert, A.K. & Muller, J.K. (1997). Biparental care and social evolution in burying beetles: lessons from the larder. In *The Evolution of Social Behavior in Insects and Arachnids* (eds J. Choe & B. Crespi), pp. 216–236. Cambridge University Press, Cambridge.

Gadgil, M. & Bossert, W.H. (1970) Life historical consequences of natural selection. *American Naturalist*, **104**, 1-24.

Hirschfield, M.F. & Tinkle, D.W. (1975) Natural selection and the evolution of reproductive effort. *Proceedings of the National Acadamy of Science USA*, **72**, 2227-2231.

Kirkwood, T.B.L. & Rose, M.R. (1991) Evolution of senescence: late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society of London B-Biological Sciences*, **332**, 15-24.

Kruuk, L.E.B., Clutton-Brock, T.H., Albon, S.D., Pemberton, J.M., & Guinness, F.E. (1999) Population density affects sex ratio variation in red deer. *Nature*, **399**, 459-461.

Lack, D. (1947) The significance of clutch size. *Ibis*, **89**, 302-352.

Lock, J.E., Smiseth, P.T., Moore, P.J., & Moore, A.J. (2007) Coadaptation of prenatal and postnatal maternal effects. *American Naturalist*, **170**, 709-718.

Marrow, P., McNamara, J.M., Houston, A.I., Stevenson, I.R., & Clutton-Brock, T.H. (1996) State-dependent life history evolution in Soay sheep: Dynamic modelling of reproductive scheduling. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **351**, 17-32.

McElligott, A.G., Altwegg, R., & Altwegg, T.J. (2002) Age-specific survival and reproductive probabilities: evidence for senescence in male fallow deer (*Dama dama*). *Proceedings of the Royal Society of London B-Biological Sciences*, **269**, 1129-1137.

McNamara, J.M. & Houston, A.I. (1996) State-dependent life histories. *Nature*, **380**, 215-221.

McNamara, J.M., Houston, A.I., Barta, Z., Scheuerlein, A., & Fromhage, L. (2009) Deterioration, death and the evolution of reproductive restraint in late life. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 4061-4066.

Monaghan, P., Metcalfe, N.B., & Torres, R. (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters*, **12**, 75-92.

Muller, J.K., Braunisch, V., Hwang, W.B., & Eggert, A.K. (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, **18**, 196-203.

Nelson, J.B. (2005) *Pelicans, cormorants and their relatives: The Pelecaniformes* Oxford University Press, Oxford.

Nussey, D.H., Kruuk, L.E.B., Morris, A., Clements, M.N., Pemberton, J.M., & Clutton-Brock, T.H. (2008) Inter- and intrasexual variation in aging patterns across reproductive traits in a wild deer population. *American Naturalist*, **174**, 342-357.

Pärt, T., Gustafsson, L., & Moreno, J. (1992) "Terminal investment" and a sexual conflict in the collared flycatcher (*Ficedula abicollis*). *American Naturalist*, **140**, 868-882.

Partridge, L. (2010) The new biology of ageing. *Philosophical Transactions of the Royal Society of London B-Biological Sciences*, **365**, 147-154.

534 Partridge, L. & Gems, D. (2006) Beyond the evolutionary theory of ageing, from functional
535 genomics to evo-gero. *Trends in Ecology & Evolution*, **21**, 334-340.
536 Plaistow, S.J., Outreman, Y., Moret, Y., & Rigaud, T. (2003) Variation in the risk of being
537 wounded: an overlooked factor in studies of invertebrate immune function? *Ecology*
538 *Letters*, **6**, 489-494.
539 Povey, S.R., Cotter, S.C., Simpson, S.J., Lee, K., & Wilson, K. (2009) Can the protein costs
540 of bacterial resistance be offset by altered feeding behaviour? *Journal of Animal*
541 *Ecology*, **78**, 437-446.
542 Pugesek, B.H. (1981) Increased reproductive effort with age in the California gull (*Larus*
543 *californicus*). *Science*, **212**, 822-823.
544 Reaney, L.T. & Knell, R. (2010) Immune activation but not male quality affects female
545 current reproductive investment in a dung beetle. *Behavioral Ecology*, **In Press**.
546 Reid, J.M., Bignal, E.M., Bignal, S., McCracken, D.I., & Monaghan, P. (2003) Age-specific
547 reproductive performance in red-billed choughs *Pyrrhocorax pyrrhocorax*: patterns
548 and processes in a natural population. *Journal of Animal Ecology*, **72**, 765-776.
549 Rose, M.R. (1991) *The evolutionary biology of aging* Oxford University Press, New York.
550 Sadd, B., Holman, L., Armitage, H., Lock, F., Marland, R., & Siva-Jothy, M.T. (2006)
551 Modulation of sexual signalling by immune challenged male mealworm beetles
552 (*Tenebrio molitor*, L.): evidence for terminal investment and dishonesty. *Journal of*
553 *Evolutionary Biology*, **19**, 321-325.
554 Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of*
555 *Entomology*, **43**, 595-618.

556 Smiseth, P.T., Dawson, C., Varley, E., & Moore, A.J. (2005) How do caring parents respond
557 to mate loss? Differential response by males and females. *Animal Behaviour*, **69**, 551-
558 559.
559 Tavecchia, G., Coulson, T., Morgan, B.J.T., Pemberton, J.M., Pilkington, J.C., Gulland,
560 F.M.D., & Clutton-Brock, T.H. (2005) Predictors of reproductive cost in female Soay
561 sheep. *J. Anim. Ecol.*, **74**, 201-213.
562 Trumbo, S.T. (1991) Reproductive benefits and the duration of paternal care in a biparental
563 burying beetle, *Nicrophorus orbicollis*. *Behaviour*, **117**, 82-105.
564 Trumbo, S.T. (2009) Age-related reproductive performance in the parental burying beetle,
565 *Nicrophorus orbicollis*. *Behavioral Ecology*, **20**, 951-956.
566 Velando, A., Drummond, H., & Torres, R. (2006) Senescent birds redouble reproductive
567 effort when ill: confirmation of the terminal investment hypothesis. *Proceedings of*
568 *the Royal Society B-Biological Sciences*, **273**, 1443-1448.
569 Ward, R.J.S. (2007) *Ageing and reproductive strategies in the Burying Beetle Nicrophorus*
570 *vespilloides*, University of Cambridge, Cambridge.
571 Ward, R.J.S., Cotter, S.C., & Kilner, R.M. (2009) Current brood size and residual
572 reproductive value predict offspring desertion in the burying beetle *Nicrophorus*
573 *vespilloides*. *Behavioral Ecology*, **20**, 1274-1281.
574 Weil, Z.M., Martin, L.B., Workman, J.L., & Nelson, R.J. (2006) Immune challenge retards
575 seasonal reproductive regression in rodents: evidence for terminal investment. *Biology*
576 *Letters*, **2**, 393-396.
577 Williams, G., ed. (1966) *Adaptation and natural selection*. Princeton University Press,
578 Princeton, New Jersey.

Wilson, K. & Hardy, I.C.W. (2002). Statistical analysis of sex ratios: an introduction. In *Sex Ratios: Concepts and Research Methods* (ed I.C.W. Hardy), pp. 48-92. Cambridge University Press.

Tables

Table 1. Experimental treatments used to investigate how age and state influence current reproduction. Ticks indicate the treatment combinations for which data was collected. Cells outlined in black represent the data used in Figure 1, while filled grey cells represent data used in Figure 2.

		Number of previous breeding attempts				
		0	**1**	**2**	**3**	**4**
Age	**12**	✓	X	X	X	X
	24	✓	✓	X	X	X
	36	✓	✓	✓	X	X
	48	✓	✓	✓	✓	X
	60	✓	✓	✓	✓	✓

Figure legends

Figure 1. A female *Nicrophorus vespilloides* burying beetle providing care for her brood. Photo courtesy of O. Kruger.

Figure 2. The effect of age at first reproduction on the total weight of larvae produced from successful first broods. Means with different subscripted letters are significantly different from each other ($P < 0.05$).

Figure 3. The effects of prior reproductive investment on the weight of the current brood are shown for females mating at a) 24 days, b) 36 days, c) 48 days and d) 60 days. Analysis only includes data from females that successfully produced offspring in their current brood. The figures show that current brood weight decreases with age in all females and with prior reproductive investment in older females (36, 48 and 60 days old). Regression lines are predictions from REML models controlling for the random effect of female ID, whilst data points are raw data.

Figure 4. The effect of age and immune challenge treatment on a) investment in the current brood and b) the weight change experienced by breeding females.

611

612

613 Figure 1

614

615

616

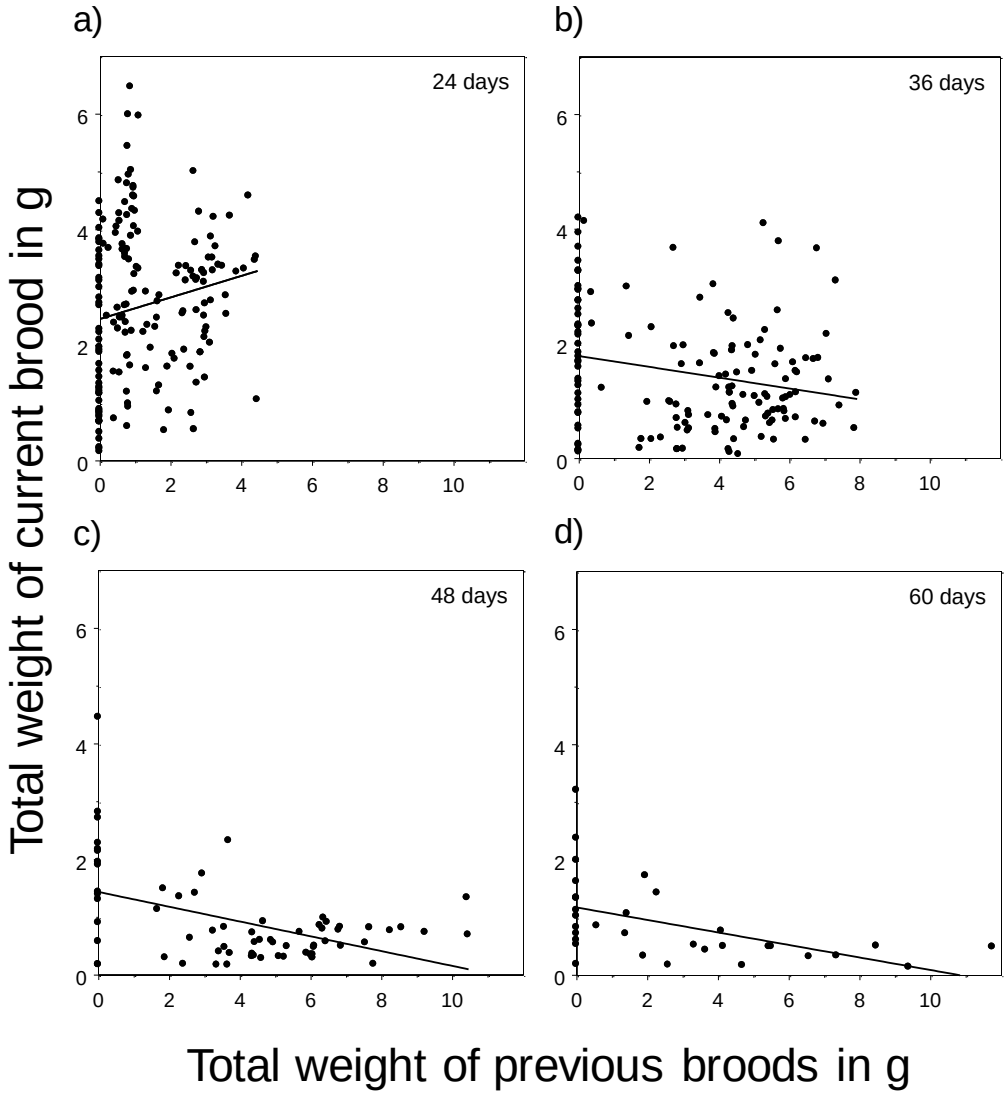
617 Figure 2

618

619

620

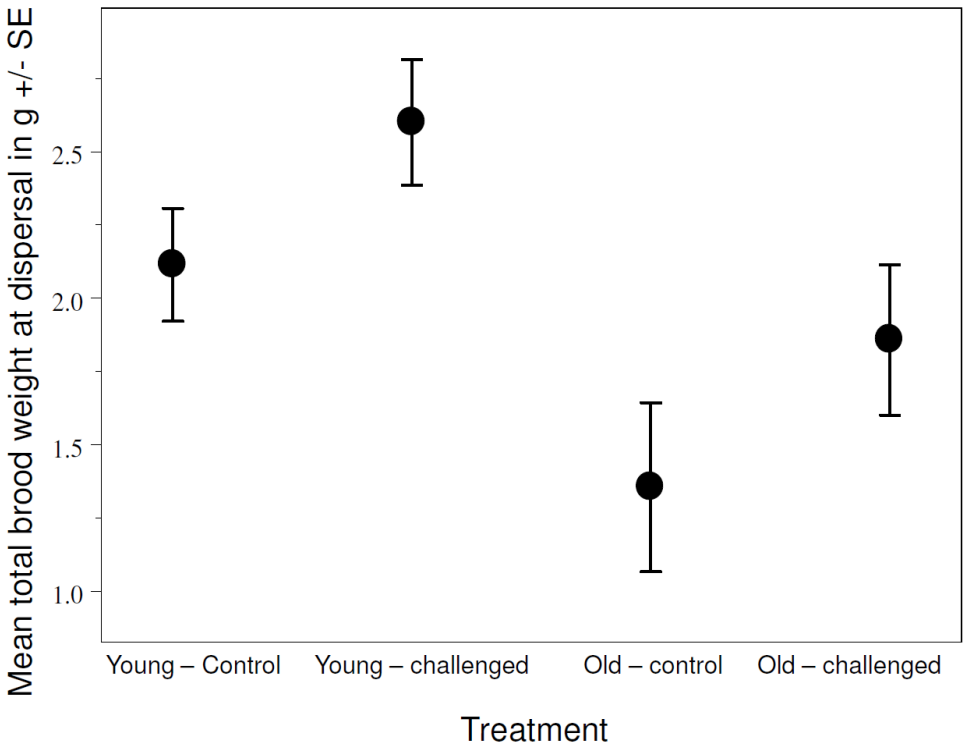
621 Figure 3



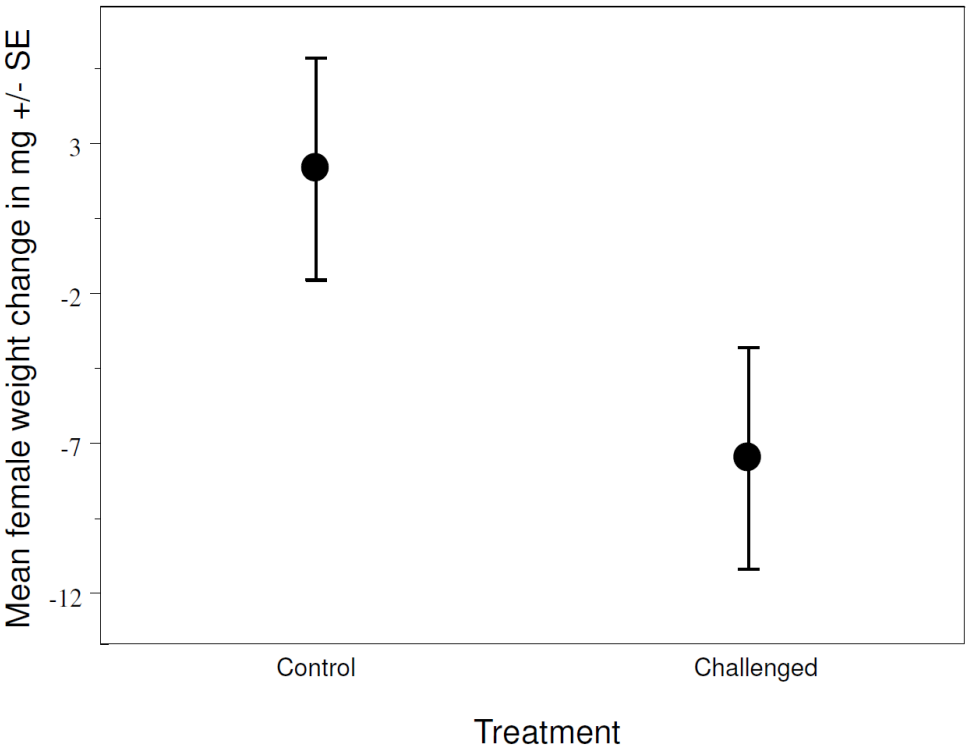
622

623 Figure 4

a)


Mean total brood weight at dispersal in g +/- SE
2.5
2.0
1.5
1.0
Young – Control
Young – challenged
Old – control
Old – challenged
Treatment

b)


Mean female weight change in mg +/- SE
3
-2
-7
-12
Control
Challenged
Treatment

624